UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	STEVENS, Fred J. et. al.
Title:	FIBRIL-BLOCKING PEPTIDE, A METHOD FOR PREVENTING FIBRIL FORMATION
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Jillian Szafranski — Name of Representative

Jillian Szafranski — Signature

7/17/2003 — Date of Signature

Commissioner for Patents
Alexandria, VA 22313

20 N. Wacker Drive
Chicago, IL 60606
(312) 621-1330

AMENDMENT TO CLAIMS

Sir:

In response to the July 2, 2003 Notice to Comply with the Sequence Rules in the above-identified matter, applicant requests entry of the amendment to claim 13. The claims as amended to date are listed in their entirety beginning on the following sheet.

CLAIMS

1. (Previously Amended) A method for minimizing the aggregation tendencies of an amyloid forming protein, the method comprising:

a) identifying SMA or LEN mutation in the amino acid sequence of said protein that leads to fibril formation;

b) substituting each mutation into SMA or LEN to identify the residues of a peptide that contribute to fibril formation;

c) synthesizing peptides spanning most of the light chain variable region that interacts with an endoplasmic reticulum chaperone selected from the group consisting of BiP, Hsp 70, and combinations thereof;

d) determining the V_L-derived peptides for their ability to prevent fibril formation in vitro wherein the peptides are selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS (SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), LTLKLSR (SEQ ID NO: 13) and combinations thereof; and

e) preventing fibril formation by inserting the said peptide into the complimentary region of the light chain variable domain.

2. (Previously Amended) The method as recited in claim 1 wherein the method is conducted in a cell.

3. (Previously Amended) The method as recited in claim 1 wherein the protein is human kappa-4 light chain variable domain or a greek key fold protein selected from the group consisting of antibody constant domains, transthyretin, beta-2 microglobulin, serine protease inhibitors, and crystalline.

4. (Previously Amended) The method as recited in claim 3 wherein the peptide is an amino acid sequence identical to an amino acid sequence in a region of the light chain variable domain.

5. (Previously Amended) The method as recited in claim 3 wherein the peptide is inserted between residue position numbers 60 and 83 of the human kappa-IV light chain.

6. (Previously Amended) The method as recited in claim 3 wherein the peptide is the amino acid sequence Phe_{71}-Thr_{72}-Leu_{73}-Thr_{74}-Ile_{75}-Ser_{76}-Ser_{77} (SEQ ID NO: 1) and wherein the subscripts denote the positions of the amino acids in the domain.

7. (Previously Amended) The method as recited in claim 1 wherein the peptide is inserted when the amyloid forming protein is partially unfolded.

8. (Original) The method as recited in claim 1 wherein the peptide is identical in composition to a portion of the protein that anchors a hairpin-shaped amino acid sequence to the protein.

9. (Original) The method as recited in claim 1 wherein the protein is a greek key fold protein selected from the group consisting of antibody constant domains, transthyretin, beta-2-microglobulin, serine protease inhibitors, and crystalline.

10. (Previously Amended) The method as recited in claim 9 wherein the peptide is inserted at a hairpin anchorage point in the human kappa-IV protein and its derivatives selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS

(SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), LTLKLSR (SEQ ID NO: 13), and combinations thereof.

11. (Original) The method as recited in claim 1 wherein the peptide is a target for an endoplasmic reticulum chaperone.

12. (Previously Amended) The method as recited in claim 1 wherein the peptide is an endoplasmic reticulum chaperone selected from the group consisting of hsp70 and BiP.

13. (Currently Amended) The method as recited in claim 1 wherein the peptide interacts with endoplasmic reticulum chaperone, the peptide selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS (SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), and LTLKLSR (SEQ ID NO: 13).

14. (Original) A peptide for insertion in an intact human kappa-IV light chain variable domain, the peptide comprising the following amino acid sequence:

Phe_{71}-Thr_{72}-Leu_{73}-Thr_{74} -Ile_{75}-Ser_{76}-Ser_{77}

wherein the subscript numbers are the residue location points in the domain.

15. (Original) A method for preventing amyloid formation in human kappa-IV light chain variable domain, the method comprising inserting the peptide Phe_{71}-Thr_{72}-Leu_{73}-Thr_{74} -Ile_{75}-Ser_{76}-Ser_{77} into the domain, wherein the subscript numbers indicate the residue location on the domain.

16. (Original) The method as recited in claim 15 wherein the domain is partially unfolded at the time of insertion.

17. (Previously Amended) A method for preventing fibril assembly of human kappa-IV immunoglobulin, the method comprising:

a) identifying the mutations LEN and SMA in the amino acid sequences of human kappa-IV immunoglobulin;

b) substituting each SMA mutation into LEN to identify the residues of the peptide that contribute to fibril formation;

c) synthesizing peptides selected form the group consisting of those peptides spanning most of the variable region of the light chain that interacts with an endoplasmic reticulum chaperone selected from the group consisting of BiP and Hsp 70; and

d) determining the V_L-derived peptides selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS (SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), LTLKLSR (SEQ ID NO: 13), and combinations thereof for their ability to prevent fibril formation.

18. (Previously Amended) The method as recited in claim 17 wherein the protein involved in fibril assembly is human kappa-IV immunoglobulin light chains.

19. (Previously Amended) The method as recited in claim 17 wherein the binding protein binds with the region.

20. (Previously Amended) The method as recited in claim 17 wherein the binding protein is an amino acid sequence that is the same as the amino acid sequence of the region.

21. (Previously Added) Method for minimizing the aggregation tendencies of human kappa-4 immunoglobulin light chain *in vitro*, the method comprising:

a) identifying the LEN and SMA mutation in the amino acid sequence of said

protein;

b) substituting each SMA mutation into LEN to identify the residues of a peptide that contributes to fibril formation;

c) synthesizing peptides spanning most of the variable region of the light chain that interacts with an endoplasmic reticulum chaperone selected from the group consisting of BiP and Hsp 70;

d) determining the V_L-derived peptides for their ability to prevent SMA fibril formation in vitro wherein the peptides are selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS (SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), LTLKLSR (SEQ ID NO: 13), and combinations thereof.

22. (Previously Added) A method for minimizing the aggregation tendencies of human kappa-4 immunoglobulin light chain protein in a cell, the method comprising:

a) identifying the LEN and SMA mutation in the amino acid sequence of said protein;

b) substituting each SMA mutation into LEN to identify the residues of a peptide that contribute to fibril aggregation;

c) synthesizing peptides spanning most of the variable region of the light chain that interacts with an endoplasmic reticulum chaperone selected from the group consisting of BiP and Hsp 70;

d) expressing SMA or LEN in COS cells;

e) treating said cells with said peptides selected from the group consisting of TDFTLTI (SEQ ID NO: 5), FTLTISS (SEQ ID NO: 1), FTLKISR (SEQ ID NO: 6), FTLEISR (SEQ ID NO: 12), LTLKLSR (SEQ ID NO: 13), and combinations thereof; and

f) determining the V_L-derived peptides for their ability to prevent SMA fibril aggregation in said cell by western blotting or immunoflourosence.